

AD-A228 461

0038-9153/90/6503-0151\$02.00/0  
STAIN TECHNOLOGY  
Copyright © 1990 by Williams & Wilkins

DTIC FREE COPY

Vol. 65, No. 3  
Printed in U.S.A.

# VISUAL ENHANCEMENT OF MYELINATED TISSUES IN THE CENTRAL NERVOUS SYSTEM OF THE RAT USING SUDAN BLACK B.

KAREN R. OLSON AND RICHARD K. TRAUB, *Neurotoxicology Branch,  
Pathophysiology Division, U.S. Army Medical Research Institute of Chemical  
Defense, APG, Maryland 21010-5425*

DTIC  
ELECTR  
NOV 13 1990

B

D

We investigated the quantitative distribution of radiolabelled compounds in the central nervous system of the rat using autoradiographic techniques (Traub 1985). To complement our autoradiographic images, we searched for a suitable fiber stain delineating myelinated structures using fresh frozen tissue. Surprisingly, the majority of staining procedures were exclusive to fixed tissues (Lillie 1977, Lillie and Fullmer 1976). These techniques applied to fresh frozen brain tissue produced poor differentiation of myelinated and nonmyelinated areas and were generally unsatisfactory. Therefore, we performed a series of studies using lipid soluble dyes such as oil red O, Luxol fast blue, hematoxylin and Nile blue in an effort to enhance differentiation of myelin. We found Sudan black B to be superior for our purpose. The procedure we developed to define myelinated structures is described below.

**Materials and methods.** Fresh frozen rat brain sections cut at 20  $\mu$ m were thaw mounted on acid cleaned glass slides and air dried overnight. A saturated solution of Sudan stain was prepared by mixing 2 g of Sudan black B (C.I. 26150) with 100 ml of tetrahydrofuran (THF). Plastic staining dishes were used because this solution severely stains glassware with prolonged use. (Note: This procedure yields the best results when slides are hand held and stained one at a time.)

1. Immerse tissue portion of slide in Sudan stain for approximately 8 sec without movement. Optimization of immersion time may be required depending on tissue type and section thickness. It is suggested that practice slides be tested to determine times which yield sufficiently stained structures.

2. Move slide through 50% ethyl alcohol rapidly (approximately 1 sec).

3. Plunge slide into 90 C ( $\pm$ 5 C) tap water for 10 sec moving the slide back and forth through the water. Change water after every 50 slides to prevent accumulation of undissolved stain.

4. Repeat step 3 with room temperature water.

5. Before coverslipping with Kaiser glycerol gelatin (Lillie and Fullmer 1976, p. 119), wipe the slide clean of excess stain around the section with a damp, lint-free cloth. Ring the coverslip with clear nail polish.

**Results and discussion.** Staining results are shown in Fig. 1. THF was chosen as the solvent because the Sudan dye and tissue lipids are soluble in it and because it has a low boiling point (65 C). Since THF is a solvent, this procedure was performed in a draft chamber. The 50% ethyl alcohol removes the excess stain from the surface of the section and is the first step in differentiation.

DISTRIBUTION STATEMENT A

Approved for public release  
Distribution Unlimited

90 11 8 096



FIG. 1. A 20  $\mu$ m thick fresh frozen rat brain section, thaw mounted on acid cleaned slide, air dried and subsequently stained with Sudan black B. Darkly stained areas are myelinated structures. Sudan black B also stains basement membranes and probably contributes to the staining of the choroid plexus as seen above.  $\times 8$ .

The alcohol does not penetrate lipid structures as rapidly as THF, thus preserving differential lipid staining.

The second part of the process, hot tap water, flushes the THF, causing the dye to remain in myelin-rich areas. This step is essential for complete differentiation. Room temperature tap water brings the temperature of the tissue down quickly and prevents dye diffusion into nonlipid areas. It also maintains integrity of the tissue by preventing distortion due to heat. It is possible to differentiate further by a quick dip in 100% ethyl alcohol, followed by a rinse with room temperature tap water. The dye is removed quickly in this manner. Perform this differentiation step with care. Once the dye is removed by alcohol, the tissue will be impervious to stain because the alcohol also removes lipids.

Dye may be observed to accumulate on the sections as small round clusters. This may be due to a supersaturated Sudan solution. Although some undissolved dye is acceptable, this may be a problem and can be corrected by adding a small amount of THF. It is important to mention that THF evaporates during normal use so the quantity of undissolved dye constantly increases. Reconstitute the solution with fresh THF as needed. Air currents produced by the swift movement from the stain to alcohol to hot water contribute to

the formation of dye crystals. Holding the slide with the section facing away from the air current as it is transferred will greatly reduce dye precipitation. It is also important to use hot tap water within 5° C of the stated temperature. If the water is too cool, THF will not vaporize from the tissue and poor differentiation will result. If the water is boiling, the tissue will be destroyed. Isopropyl alcohol can be substituted for ethanol, but methyl alcohol should not be used because it blisters the tissue.

This staining technique is satisfactory at low magnifications for defining fiber bundles and myelinated areas. However, at higher magnifications, the stain is inadequate. The resolution of these areas is poor, and tiny crystal accumulations restrict the crisp image of the tissue that is seen at the lower powers.

Counterstaining with (0.05%) neutral red for 30 sec gives good definition of cell nuclei in contrast to the fibered areas, and this dual staining becomes very useful for identifying areas of interest. It is important to stress that after counterstaining with neutral red, a wash with distilled water followed by mounting as discussed in step 5, gives the best results.

Slides stained with only Sudan black B keep well for more than five years and do not fade.

## REFERENCES

- Lillie, R. D. (Ed.) 1977. *H. J. Conn's Biological Stains*, 9th ed. Williams & Wilkins, Baltimore, p. 577.
- Lillie, R. D. and Fullmer, H. M. 1976. *Histopathologic Technic and Practical Histochemistry*, 4th ed. McGraw-Hill, New York, p. 571.
- Traub, K. 1985. *In vivo* distribution of <sup>14</sup>C radiolabeled soman ((3,3-dimethyl-2-butoxy)-methylphosphorylfluoride) in the central nervous system of the rat. *Neurosci. Lett.* 60: 219-225.



Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	20

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a. REPORT SECURITY CLASSIFICATION <u>UNCLASSIFIED</u>			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT  <u>Approved for public release; unlimited</u>		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			4. PERFORMING ORGANIZATION REPORT NUMBER(S)  <u>P89-057</u>		
5. MONITORING ORGANIZATION REPORT NUMBER(S)  <u>P89-057</u>			6a. NAME OF PERFORMING ORGANIZATION <u>US Army Medical Research Institute of Chemical Defense SGRD-UV-YN</u>		
6b. OFFICE SYMBOL (if applicable)			7a. NAME OF MONITORING ORGANIZATION <u>US Army Medical Research Institute of Chemical Defense SGRD-UV-RC</u>		
6c. ADDRESS (City, State, and ZIP Code)  <u>Aberdeen Proving Ground, MD 21010-5425</u>			7b. ADDRESS (City, State, and ZIP Code)  <u>Aberdeen Proving Ground, MD 21010-5425</u>		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION			8b. OFFICE SYMBOL (if applicable)		
8c. ADDRESS (City, State, and ZIP Code)			9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER		
10. SOURCE OF FUNDING NUMBERS			11. TITLE (Include Security Classification)		
PROGRAM ELEMENT NO. <u>61102A</u>			PROJECT NO. <u>3M161102B</u>		
TASK NO. <u>511-AA</u>			WORK UNIT ACCESSION NO.		
12. PERSONAL AUTHOR(S) <u>Karen R. Olson and Richard K. Traub</u>			13. TYPE OF REPORT <u>Open Literature Publ</u>		
13a. TIME COVERED FROM _____ TO _____			14. DATE OF REPORT (Year, Month, Day)		
15. PAGE COUNT <u>3</u>			16. SUPPLEMENTARY NOTATION  <u>Stain Technology 1990</u>		
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD			GROUP		
SUB-GROUP			fiberstain Sudan Black B		
			Myelinated tissue		
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
<p><i>This Report</i></p> <p>We investigated the quantitative distribution of radiolabelled compounds in the central nervous system of the rat using autoradiographic techniques (Traub 1985). To complement our autoradiographic images, we searched for a suitable fiber stain delineating myelinated structures using fresh frozen tissue. Surprisingly, the majority of staining procedures were exclusive to fixed tissues (Little 1977, Little and Fullmer 1970). These techniques applied to fresh frozen brain tissue produced poor differentiation of myelinated and nonmyelinated areas and were generally unsatisfactory. Therefore, we performed a series of studies using lipid soluble dyes such as oil red O, Luxol fast blue, hematoxylin and Nile blue in an effort to enhance differentiation of myelin. We found Sudan black B to be superior for our purpose. The procedure we developed to define myelinated structures is described below.</p> <p style="text-align: right;">(772)</p>					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION		
22a. NAME OF RESPONSIBLE INDIVIDUAL <u>Wade, John V., LTC</u>			22b. TELEPHONE (Include Area Code) <u>301-671-2553</u>		
			22c. OFFICE SYMBOL <u>SGRD-UV-YN</u>		

DD Form 1473, JUN 86

Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED